transmembrane domain extends from amino acid Leu-220 to Ala-245. The deduced extracellular domain sequence includes amino acids Gly-27 to Pro-219.--

Please replace the paragraph beginning at page 6, line 5, with the following rewritten paragraph:

B2 --Figure 2A-2D shows the AL-2s-encoding nucleotide sequence (SEQ ID NO: 3), its complementary sequence, and the deduced amino acid sequence (SEQ ID NO: 4) of AL-2 of the isolated AL-2s ("AL-2-short") cDNA. The deduced N-terminus of the mature AL-2 protein begins with glycine-27 as numbered from the initiation methionine. The C-terminal hydrophobic transmembrane domain extends from amino acid Leu-220 to Ala-245. The deduced extracellular domain sequence includes amino acids Gly-27 to Pro-219.--

Please replace the paragraph beginning at page 6, with the following rewritten paragraph:

B3 --Figure 3A-3C depicts an alignment of the AL-2l nucleotide sequence with human EST sequence H10006 (SEQ ID NO: 5).--

Please replace the paragraph beginning at page 6, line 12, with the following rewritten paragraph:

B4 --Figure 4A-4C shows a comparison of the AL-2l and AL-2s amino acid sequences with that of Lerk2 (SEQ ID NO: 9) (Beckmann *et al., EMBO J.,* 13:3757-3762 (1994)) and human Htk-L (SEQ ID NO: 10) (Bennett *et al., Proc. Natl. Acad. Sci. USA,* 92:1866-70 (1995); WO 96/02645 published February 1, 1996; both are incorporated by reference herein). Identical amino acids are boxed, and gaps introduced for optimal alignment are indicated by dashes. Conserved cysteine residues can be seen. The deduced C-terminal amino acid AL-2s is valine.--

Please replace the paragraph beginning at page 6, line 17, with the following rewritten paragraph:

B5 --Figure 5A-5B shows a comparison of the AL-2l amino acid sequences with that of Lerk2 (SEQ ID NO: 9) and human Htk-L (SEQ ID NO: 10). Identical amino acids are boxed, and gaps introduced for optimal alignment are indicated by dashes. Conserved cysteine residues can be seen.--

Please replace the paragraph beginning at page 6, line 22, with the following rewritten paragraph:

--"AL-2" or "AL-2 protein" refers to a polypeptide or protein encoded by the AL-2 nucleotide sequence set forth in Figures 1A-1C (showing AL-2l) or 2 (showing AL-2s); a polypeptide that is the translated amino acid sequence set forth in Figures 1A-1C or 2A-2D; fragments thereof having greater than about 5 contiguous amino acid residues and comprising an immune epitope or other biologically active site of AL-2; amino acid sequence variants of the amino acid sequence set forth in Figures 1A-1C or 2A-2D wherein one or more amino acid residues are added at the N- or C-terminus of, or within, said Figures 1A-1C or 2A-2D sequences or its fragments as defined above; amino acid sequence variants of said Figures 1A-1C or 2A-2D sequences or its fragments as defined above wherein one or more amino acid residues of said Figures 1A-1C or 2A-2D sequences or fragment thereof are deleted, and optionally substituted by one or more amino acid residues; and derivatives of the above proteins, polypeptides, or fragments thereof, wherein an amino acid residue has been covalently modified so that the resulting product is a non-naturally occurring amino acid. Preferred embodiments retain a biologically property of AL-2. AL-2 amino acid sequence variants may be made synthetically, for example, by site-directed or PCR mutagenesis, or may exist naturally, as in the case of allelic forms and other naturally occurring variants of the translated amino acid sequence set forth in Figures 1A-1C or 2A-2D that occur in human or other animal species. Accordingly, within the scope of the present invention are AL-2 proteins derived from other animal species, preferably mammalian, including but not limited to murine, rat, bovine, porcine, or various primates. As used herein, the term "AL-2" includes membrane-bound proteins (comprising a cytoplasmic domain, a transmembrane region, and an extracellular domain), including the long and short forms of AL-2, as well as truncated proteins that retain Eph-family-receptor binding property. Truncated AL-2 proteins include, for example, soluble AL-2 comprising only the extracellular (receptor binding) domain. Such fragments, variants, and derivatives exclude any polypeptide heretofore identified, including any known neurotrophic factor, such as nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), Eph family receptor ligand such as Erk-L or Lerk-2, as well as statutorily obvious variants thereof. A preferred AL-2 is one having a contiguous amino acid sequence of or derived from mature AL-2 shown in Figures 1A-1C or 2A-2D.--

B6

Please replace the paragraph beginning at page 8, line 8, with the following rewritten paragraph:

--Biologically active or antigenically active AL-2 polypeptides embodiments of this invention include the polypeptide represented by the entire translated nucleotide sequence of AL-2l and Al-2s (including their signal sequence); mature AL-2, *i.e.,* AL-2 without the signal sequence; fragments consisting essentially of the intracellular domain or transmembrane domain of AL-2; fragments of the AL-2 having a contiguous sequence of at least 5, 10, 15, 20, 25, 30, or 40 consecutive amino acid residues from AL-2; amino acid sequence variants of AL-2 wherein an amino acid residue has been inserted N- or C-terminal to, or within, AL-2 or its fragment as defined above; amino acid sequence variants of AL-2 or its fragment as defined above wherein an amino acid residue of Al-2 or its fragment as defined above has been substituted by another residue, including predetermined mutations by, *e.g.,* site-directed or PCR mutagenesis, AL-2 of various animal species such as rabbit, rat, porcine, non-human primate, equine, murine, and ovine AL-2 and alleles or other naturally occurring variants of the foregoing and human AL-2; derivatives of AL-2 or its fragments as defined above wherein AL-2 or its fragments have been covalent modified, by substitution, chemical, enzymatic, or other appropriate means, with a moiety other than a naturally occurring amino acid; and glycosylation variants of AL-2 (insertion of a glycosylation site or alteration of any glycosylation site by deletion, insertion, or substitution of suitable residues). The preferred AL-2 is human AL-2, especially native human AL-2 having the sequence shown in Figures 1A-1C or 2A-2D.--

B1

Please replace the paragraph, beginning at page 8, line 24, with the following rewritten paragraph:

--One embodiment of the present invention provides soluble AL-2. By "soluble AL-2" is meant AL-2 which is essentially free of at least a transmembrane sequence and, optionally, the intracellular domain of native AL-2. By "essentially free" is meant that the soluble AL-2 sequence has less than 2% of the transmembrane domain, preferably less than 1% of the transmembrane domain, and more preferably less than 0.5% of this domain. The transmembrane domain of the native human mature amino acid sequences are delineated in Figures 1A-1C and 2A-2D (for AL-2l and AL-2s, respectively), *i.e.*, resides Gly-27 to Pro-219. Soluble AL-2s have

B8

therapeutic advantages because they are generally soluble in the patient's blood stream. Similarly, soluble ligand may prove to be particularly useful as diagnostics since they are expected to have a reduced tendency to incorporate in the cell membrane. Soluble AL-2 polypeptides comprise all or part of the extracellular domain of a native AL-2 but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. Soluble AL-2 polypeptides advantageously comprise the native (or a heterologous) signal peptide when initially synthesized to promote secretion, but the signal peptide is cleaved upon secretion. In preferred embodiments, the soluble AL-2 polypeptides retain the ability to bind an Eph-family receptor with preferences as discussed herein. Soluble AL-2 can also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble AL-2 protein is capable of being secreted or otherwise isolated.--

B8 cont.

Please replace the paragraph, beginning at page 9, line 7, with the following rewritten paragraph:

--In one embodiment a soluble AL-2 is an "immunoadhesin". The term "immunoadhesin" is used interchangeably with the expression "AL-2-immunoglobulin chimera" and refers to a chimeric molecule that combines the extracellular domain ("ECD") of AL-2 with an immunoglobulin sequence. The immunoglobulin sequence preferably, but not necessarily, is an immunoglobulin constant domain. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG-1, IgG-2, IgG-3 or IgG-4 subtypes, IgA, IgE, IgD or IgM, but preferably IgG-1 or IgG-3. The expression "extracellular domain" or "ECD" when used herein refers to any polypeptide sequence that shares a receptor binding function of the extracellular domain of the naturally occurring AL-2 disclosed herein. Receptor binding function refers to the ability of the polypeptide to bind the extracellular domain of a Eph-family receptor, with preferences as discussed herein, and optionally, activate the receptor. Accordingly, it is not necessary to include the entire extracellular domain since smaller segments are commonly found to be adequate for receptor binding. The term ECD encompasses polypeptide sequences in which the cytoplasmic domain and hydrophobic transmembrane sequence (and, optionally, 1-20 amino acids amino-terminal to the transmembrane domain) of the mature AL-2 have been deleted. The extracellular domain sequence of AL-2 is provided in Figures 1A-1C and 2A-2D.--

B9

Please replace the paragraph, beginning at page 10, line 3, with the following rewritten paragraph:

--An AL-2 amino acid sequence variant is included within the scope of the invention provided that it is functionally active. As used herein, "functionally active" and "functional activity" in reference to Al-2 for the purposes herein means an *in vivo* effector or antigenic function or activity that is performed by Al-2 of the sequence in Figures 1A-1C or 2A-2D (whether in its native or denatured conformation). A principal effector function is the ability of AL-2 to bind to, and/or activate, a receptor from the Eph-receptor family, preferably a receptor for the transmembrane-ligand family that is also, more preferably, a human receptor. Less preferred are their non-human homologs.--

B10

Please replace the paragraph beginning at page 10, line 10, with the following rewritten paragraph:

--Generally, the ligand will bind to the extracellular domain of the receptor and thereby activate its intracellular tyrosine kinase domain. Consequently, binding of the ligand to the receptor can result in enhancement or inhibition of proliferation and/or differentiation and/or activation of cells having a receptor for AL-2 *in vivo, ex vivo,* or *in vitro.* Other effector functions include signal transduction, any enzyme activity or enzyme modulatory activity (*e.g.*, tyrosine kinase activity), or any structural role, for example. An antigenic function means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against the polypeptide sequence of a naturally occurring polypeptide comprising the polypeptide sequences of Figures 1A-1C and 2A-2D.--

B11

Please replace the paragraph beginning at page 10, line 18, with the following rewritten paragraph:

--In preferred embodiments, antigenically active AL-2 is a polypeptide that binds with an affinity of at least about 10^6 l/mole to an antibody capable of binding AL-2. Ordinarily, the polypeptide binds with an affinity of at least about 10^7 l/mole. In particular, an AL-2 is able to promote or enhance the growth, survival, function, activation, and/or differentiation of neurons and glia, whether the neurons be central, peripheral, motoneurons, or sensory neurons, *e.g.*, photoreceptors, vestibular ganglia, spinal ganglia, auditory hair cells, and the AL-2 is

B12

immunologically cross-reactive with an antibody directed against an epitope of naturally occurring AL-2. Therefore, AL-2 amino acid sequence variants generally will share at least about 75% (preferably at least 80%, more preferably at least 90%, even more preferably at least 95%, with increasing preference to at least 99%, and finally 100%) sequence identity with the translated amino acid sequence set forth in Figures 1A-1C and 2A-2D, after aligning the sequences and introducing gaps, if necessary, to achieve maximal percent identity. This is typically determined, for example, by the Fitch, *et al., Proc. Nat. Acad. Sci. USA,* 80:1382-1386 (1983), version of the algorithm described by Needleman, *et al., J. Mol. Biol.,* 48:443-453 (1970). None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the AL-2 sequence shall be construed as affecting sequence identity or homology. Preferably, the AL-2 nucleic acid molecule that hybridizes to nucleic acid sequence encoding AL-2 contains at least 20, more preferably 40, even more preferably 70, and most preferably 90 bases. For fragments, the percent identity is calculated for that portion of a native sequence that is present in the fragment.--

B12 cont

Please replace the paragraph, beginning at page 11, line 3, with the following rewritten paragraph:

--In one embodiment an isolated AL-2 protein induced phosphorylation of an Eph-family receptor and contains an amino acid sequence selected from the group consisting of (a) the amino acid sequence for mature AL-2l, (b) the amino acid sequence for mature AL-2s, (c) the naturally occurring amino acid sequence for mature AL-2 from a non-human animal species, (d) allelic variants of the sequence of (a), (b), or (c), and (e) the sequences of (a), (b), (c), or (d) having a single preferred conservative amino acid substitution as defined in Table 1. In a preferred embodiment the phosphorylation-inducing AL-2 has the amino acid sequence for mature human AL-2 shown in Figures 1A-1C or 2A-2D. Generally the AL-2 will be a chimera, membrane or liposome bound, or epitope tagged and "clustered" (see WO 95/27060, which is incorporated herein by reference), thus mimicking its membrane-bound state and ability to induce receptor phosphorylation. In another embodiment an isolated AL-2 protein binds to the Eph-family receptor and contains an amino acid sequence selected from the group consisting of (a) the amino acid sequence for mature AL-2l, (b) the amino acid sequence for mature AL-2s, (c) the naturally occurring amino acid sequence for mature AL-2 from a non-human animal species, (d) allelic

B13

variants of the sequences of (a), (b), or (c), and (e) the sequences of (a), (b), (c), or (d) having a single preferred conservative amino acid substitution as defined in Table 1. In a preferred embodiment the AL-2 has the amino acid sequence for mature human AL-2 shown in Figures 1A-1C or 2A-2D. In another embodiment isolated soluble AL-2 binds to a Eph-family receptor and contains an amino acid sequence selected from the group consisting of (a) the amino acid sequence for mature soluble AL-2l, (b) the amino acid sequence for mature soluble AL-2s, (c) the naturally occurring amino acid sequence for mature soluble AL-2 from a non-human animal species, (d) allelic variants of the sequences of (a), (b), or (c), and (e) the sequences of (a), (b), (c), or (d) having a single preferred conservative amino acid substitution as defined in Table 1. In a preferred embodiment the soluble AL-2 has the amino acid sequence for mature soluble human AL-2 shown in Figures 1A-1C or 2A-2D. In another preferred embodiment, the soluble AL-2 is a chimeric polypeptide containing an amino acid sequence encoding mature soluble AL-2 fused to an immunoglobulin sequence. In a more preferred embodiment the chimeric polypeptide contains a fusion of an AL-2 extracellular domain sequence to an immunoglobulin constant domain sequence. Preferably the constant domain sequence is that of an immunoglobulin heavy chain. Also preferred are chimeric polypeptides containing a mature, soluble Al-2 amino acid sequence fused to an epitope tag polypeptide sequence.--

B13 cont.

Please replace the paragraph, beginning at page 12, line 25, with the following rewritten paragraph:

--Amino acid sequence variants of AL-2 are prepared by introducing appropriate nucleotide changes into AL-2 DNA and thereafter expressing the resulting modified DNA in a host cell, or by *in vitro* synthesis. Such variants include, for example, deletions from, or insertions or substitutions of, amino acid residues within the AL-2 amino acid sequence set forth in Figures 1A-1C and 2A-2D. Any combination of deletion, insertion, and substitution may be made to arrive at an amino acid sequence variant of AL-2, provided that such variant possesses the desired characteristics described herein. Changes that are made in the amino acid sequence set forth in Figures 1A-1C and 2A-2D to arrive at an amino acid sequence variant of AL-2 also may result in further modifications of AL-2 upon its expression in host cells, for example, by virtue of such changes introducing or moving sites of glycosylation, or introducing membrane

B14

B14 Cont

anchor sequences as described, for example, in PCT Pat. Pub. No. WO 89/01041 (published February 9 ,1989).--

Please replace the paragraph, beginning at page 13, line 2, with the following rewritten paragraph:

--There are two principal variables in the construction of amino acid sequence variants of AL-2: the location of the mutation site and the nature of the mutation. These are variants from the amino acid sequence set forth in Figures 1A-1C and 2A-2D, and may represent naturally occurring allelic forms of AL-2, or predetermined mutant forms of AL-2 made by mutating AL-2 DNA, either to arrive at an allele or a variant not found in nature. In general, the location and nature of the mutation chosen will depend upon the AL-2 characteristic to be modified.--

Please replace the paragraph, beginning at page 13, line 8, with the following rewritten paragraph:

--For example, due to the degeneracy of nucleotide coding sequences, mutations can be made in the AL-2 nucleotide sequence set forth in Figures 1A-1C and 2A-2D without affecting the amino acid sequence of the AL-2 encoded thereby. Other mutations can be made that will result in a AL-2 that has an amino acid sequence different from that set forth in Figures 1A-1C and 2A-2D, but which is functionally active. Such functionally active amino acid sequence variants of AL-2 are selected, for example, by substituting one or more amino acid residues in the amino acid sequence set forth in Figures 1A-1C and 2A-2D with other amino acid residues of a similar or different polarity or charge.--

B15

Please replace the paragraph, beginning at page 13, line 15, with the following rewritten paragraph:

--One useful approach is called "alanine scanning mutagenesis." Here, a an amino acid residue or group of target residues are identified (*e.g.*, charged residues such as arginine, aspartic acid, histidine, lysine, and glutamic acid) and, by means of recombinant DNA technology, replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell, (Cunningham, *et al.*, Science, 244:1081-1085 (1989)). Those domains

B16

demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at or for the sites of substitution. Obviously, such variations that, for example, convert the amino acid sequence set forth in Figures 1A-1C and 2A-2D to the amino acid sequence of a known neurotrophic factor, such as NGF, BDNF, NT-3, NT-4/5, Eph-family receptor ligand (*e.g.*, see Figures 4A-4C and 5A-5B), or another known polypeptide or protein are not included within the scope of this invention, nor are any other fragments, variants, and derivatives of the amino acid AL-2 that are not novel and unobvious over the prior art. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutations per se need not be predetermined. For example, to optimize the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed AL-2 variants are screened for functional activity.--

Please replace the paragraph, beginning at page 14, line 2, with the following rewritten paragraph:

--Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one amino acid residue to polypeptides containing a hundred or more residues, as well as intrasequences insertions of single or multiple amino acid residues. Intrasequence insertions, *i.e.*, insertions made with the amino acid sequence set forth in Figures 1A-1C or 2A-2D, may range generally from about 1 to 10 residues, more preferably 1 to 5, even more preferably 1 to 3, and most preferably 1 to 2. Examples of terminal insertions include AL-2 with an N-terminal methionyl residue (such as may result from the direct expression of AL-2 in recombinant cell culture), and AL-2 with a heterologous N-terminal signal sequence to improve the secretion of AL-2 from recombinant host cells. Such signal sequences generally will be homologous to the host cell used for expression of AL-2, and include STII or lpp for *E. coli*, alpha factor for yeast, and viral signals such as herpes gD for mammalian cells. Other insertions include the fusion to the N- or C-terminus of AL-2 of immunogenic polypeptides (for example, bacterial polypeptides such as beta-lactamase or an enzyme encoded by the *E. coli* trp locus, or yeast protein), and C-terminal fusions with proteins having a long half-life such as immunoglobulin constant regions, albumin, or ferritin, as described in PCT Pat. Pub. No. WO 89/02922 published April 6, 1989.--

Please replace the paragraph, beginning at page 14, line 16, with the following rewritten paragraph:

--The third group of variants are those in which at least one amino acid residue in the amino acid sequence set forth in Figures 1A-1C or 2A-2D, preferably one to four, more preferably one to three, even more preferably one to two, and most preferably only one, has been removed and a different residue inserted in its place. The sites of greatest interest for making such substitutions are in the regions of the amino acid sequence set forth in Figures 1A-1C or 2A-2D that have the greatest homology with other tyrosine kinase receptor ligands (for non-limiting examples, see comparisons in Figures 4 and 5). Those sites are likely to be important to the functional activity of the AL-2. Accordingly, to retain functional activity, those sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of preferred substitutions. If such substitutions do not result in a change in functional activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, may be introduced and the resulting variant AL-2 analyzed for functional activity.--

B18

Please replace the paragraph, beginning at page 15, line 23, with the following rewritten paragraph:

--Insertional, deletional, and substitutional changes in the amino acid sequence set forth in Figures 1A-1C and 2A-2D may be made to improve the stability of AL-2. For example, trypsin or other protease cleavage sites are identified by inspection of the encoded amino acid sequence for an arginyl or lysinyl residue. These are rendered inactive to protease by substituting the residue with another residue, preferably a basic residue such as glutamine or a hydrophobic residue such as serine; by deleting the residue; or by inserting a prolyl residue immediately after the residue. Also, any cysteine residues not involved in maintaining the proper conformation of AL-2 for functional activity may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.--

B19

Please replace the paragraph, beginning at page 20, line 12, with the following rewritten paragraph:

--"AL-2 nucleic acid" is RNA or DNA that encodes AL-2. "AL-2 DNA" is DNA that encodes AL-2. AL-2 DNA is obtained from cDNA or genomic DNA libraries, or by *in vitro* synthesis. Identification of AL-2 DNA within a cDNA or a genomic DNA library, or in some other mixture of various DNAs, is conveniently accomplished by the use of an oligonucleotide hybridization probe that is labeled with a detectable moiety, such as a radioisotope (Keller, *et al., DNA Probes,* pp. 149-213 (Stockton Press, 1989)). To identify DNA encoding AL-2, the nucleotide sequence of the hybridization probe preferably is selected so that the hybridization probe is capable of hybridizing preferentially to DNA encoding the AL-2 amino acid sequence set forth in Figures 1A-1C or 2A-2D, or a variant or derivative thereof as described herein, under the hybridization conditions chosen. Another method for obtaining AL-2 nucleic acid is to chemically synthesize it using one of the methods described, for example, by Engels, *et al., Agnew. Chem. Int. Ed. Engl.,* 28:716-734 (1989). A preferred embodiment is an isolated nucleic acid molecule that includes a nucleotide sequence encoding the amino acid sequence shown in Figures 1A-1C or 2A-2D for mature AL-2, and in which, more preferably, the AL-2 codons are contiguous. A preferred nucleotide sequence encoding the amino acid sequence for mature AL-2 can be found in Figures 1A-1C or 2A-2D. Also included are AL-2-encoding nucleic acid sequences based on the codon degeneracy of the genetic code.--

B20

Please replace the paragraph, beginning at page 23, line 18, with the following rewritten paragraph:

--Multiple mutations are introduced into AL-2 DNA to produce amino acid sequence variants of AL-2 comprising several or a combination of insertions, deletions, or substitutions of amino acid residues as compared to the amino acid sequence set forth in Figures 1A-1C or 2A-2D. If the sites to be mutated are located close together, the mutations may be introduced simultaneously using a single oligonucleotide that encodes all of the desired mutations. If, however, the sites to be mutated are located some distance from each other (separated by more than about ten nucleotides), it is more difficult to generate a single oligonucleotide that encodes all of the desired changes. Instead, one of two alternative methods may be employed.--

B21

Please replace the paragraph, beginning at page 63, line 27, with the following rewritten paragraph: